New alternative spectroscopic method for the detection of foodborne pathogens

Samira SARTER , Philippe DANIEL
CIRAD -UMR Qualisud
Institut des Molécules et des Matériaux du Mans
IMMM UMR CNRS 6283

cirad
LA RECHERCHE AGRONOMIQUE POUR LE DÉVELOPPEMENT

Université du Maine

cnrs

Food safety risks

Food safety risks

***Salmonella* spp.**

- Raw meat sold in market: Porc 39-64%; chicken 42-49-53%; beef 62%

- Resistance in meat: Porc 50-73% ; Chicken 45%
  - Tetracycline, sulphonamide, steptomycin, ampicillin, chloramphenicol, trimethoprim, nalidic acid

- Multiresistance : 21-56% of isolates
  - 7-9 antibiotics: 15% / 10-13 antibiotics: 8%

- Multiresistant *Salmonella* from food or food-producing animals are common in different countries:
  - Malaysia 49-75% (n=88)
  - Thailand 44-66% (n=342)
  - Vietnam 21-56% (n=180)

Thi Thu Hao Van et al. AEM 2007; Truong Ha Thai et al. IJFM 2012; Garin et al. IJFM 2012.

Food safety risks

Listeria monocytogenes

- EU rejections: Filet Pangasius (8 notifications 2010; 17 en 2009)

***Campylobacter* spp.**

- Chicken sold in market: 15.3%
- Chicken : 95% of strains are resistant to fluoroquinolones (critical AB)

***Escherichia coli* : a reservoir**

- Resistance: 84% of isolates of beef, poultry, porc
- Resistance to fluoroquinolones: 16-21% of isolates, mainly in chicken samples (52-63%)
- Multiresistant *E. coli* (n=99) in raw meat:
  - 89.5% in chicken meat
  - 95% in chicken faeces
  - 75% in pork meat isolates

Garin et al. IJFM 2012; Thi Thu Hao Van et al. IJFM 2012; Truong Ha Thai et al. IJFM 2012; Thi Thu Hao Van et al. AEM 2007; Thi Thu Hao Van et al. IJFM 2008.

Food safety risks

- **Food Safety Objectives**: "the maximum frequency and/or concentration of a hazard in a food at the time of consumption that provides or contributes to the appropriate level of protection (ALOP)".

- To ensure that an FSO is met, it is required to set **Performance Objectives** which correspond to the levels that must be met at earlier steps in the food chain before consumption.

- FSOs and POs must be achievable by the application of good practices (GAP, GHP, GMP) and HACCP

- **Microbiological Criteria** can be used to define the microbiological quality of raw materials, food ingredients, and end-products at any stage in the food chain.

Need for accurate, rapid and sensitive methods for detection and quantification of microbial hazards

Standard methods for pathogen identification

AFNOR ISO 6579:2002

Identification of *Salmonella* spp

25g of sample

Incubation

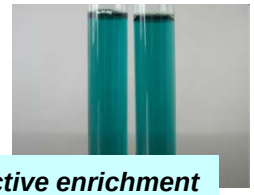
2 - 4 days

Identification

Time depending on method

Many hours

Pre enrichement
Incubation in BPW



Selective enrichment
RVS + MKTTn

Isolement
XLD + XLT4

Incubation
Agar plate

Phenotypic methods

Immunological methods (ELISA)

Molecular methods (PCR)

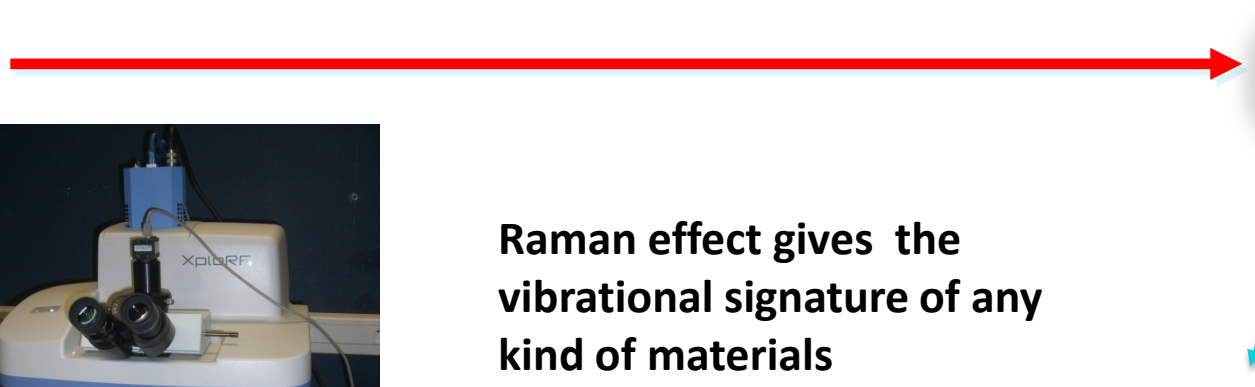
Biochemical methods

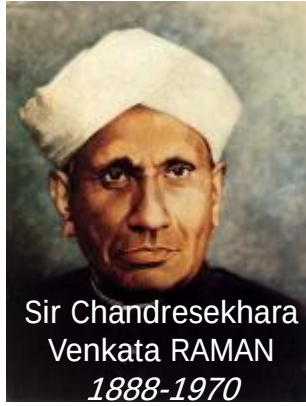
Applications of Raman spectroscopy to bacteria

Principles of Raman spectroscopy

monochromatic visible radiation : Laser ω_0, λ_0

Interaction with a sample

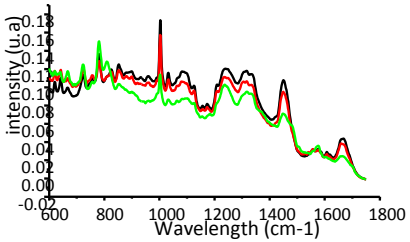




Raman effect gives the vibrational signature of any kind of materials

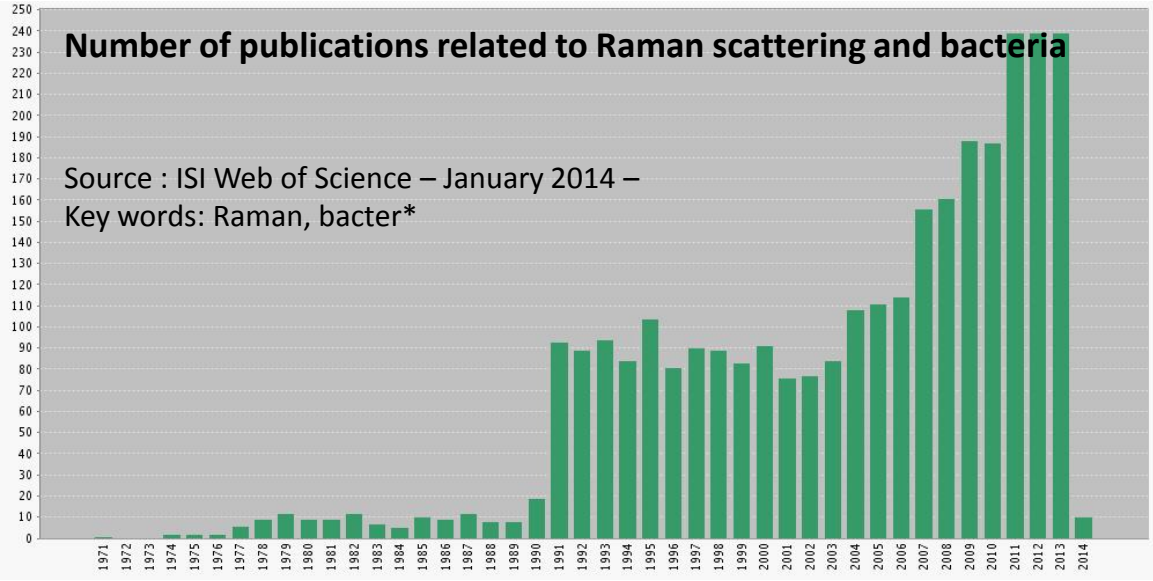
Scattered radiations

Inelastic process



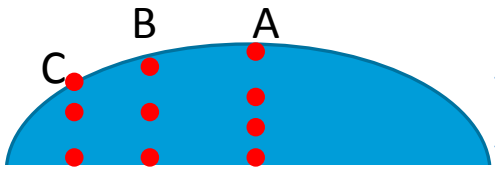
Advantages of the technics:

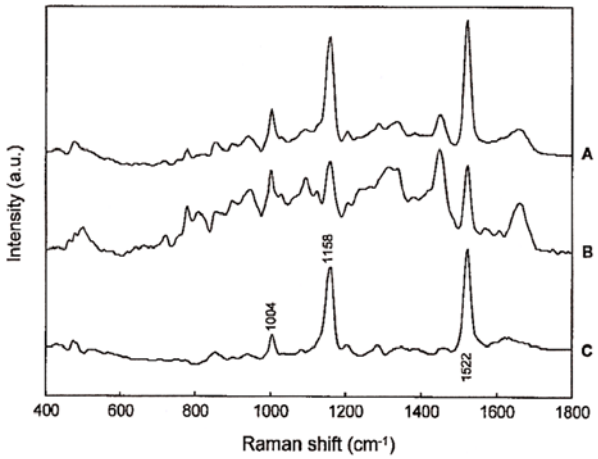
- Fingerprint technics
- No preparation of the sample
- Non invasive technics
- Non destructive technics
- Qualitative or quantitative



Raman study of bacteria

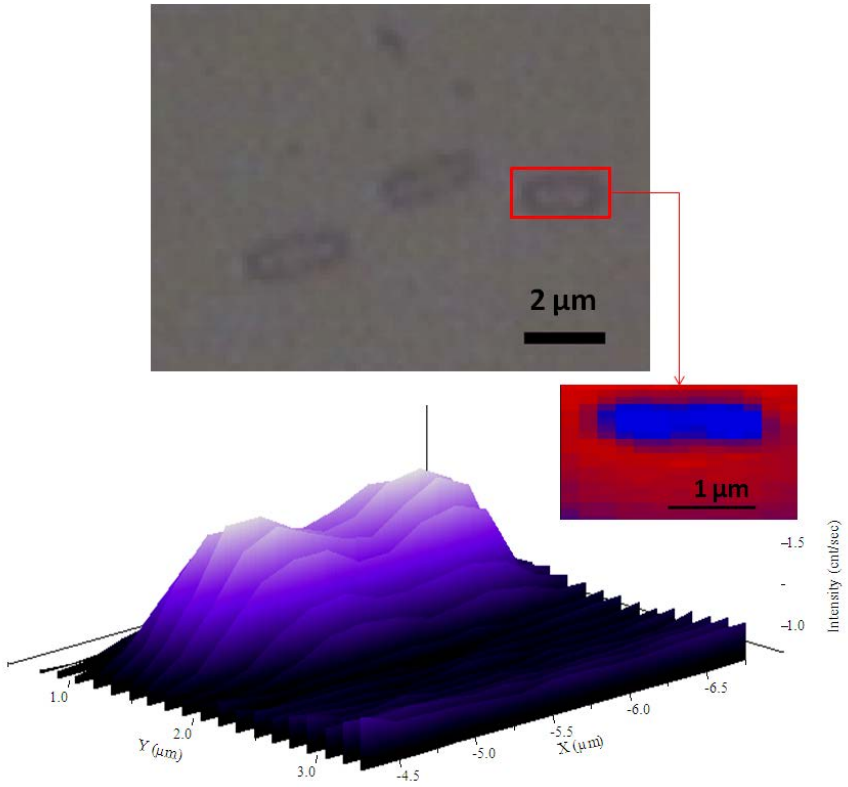
- Investigation of microcolonies and characterisation of heterogeneity





L.P. Choo-Smith et al, Applied and environmenetal microbiology, 2001

- Single-cell analysis of bacteria



Pongsit Tangcananurak
Work done in the framework of Franco-Thai Program in 2008

Raman study of bacteria

Interprétation of the spectrum: ***fingerprint technique***

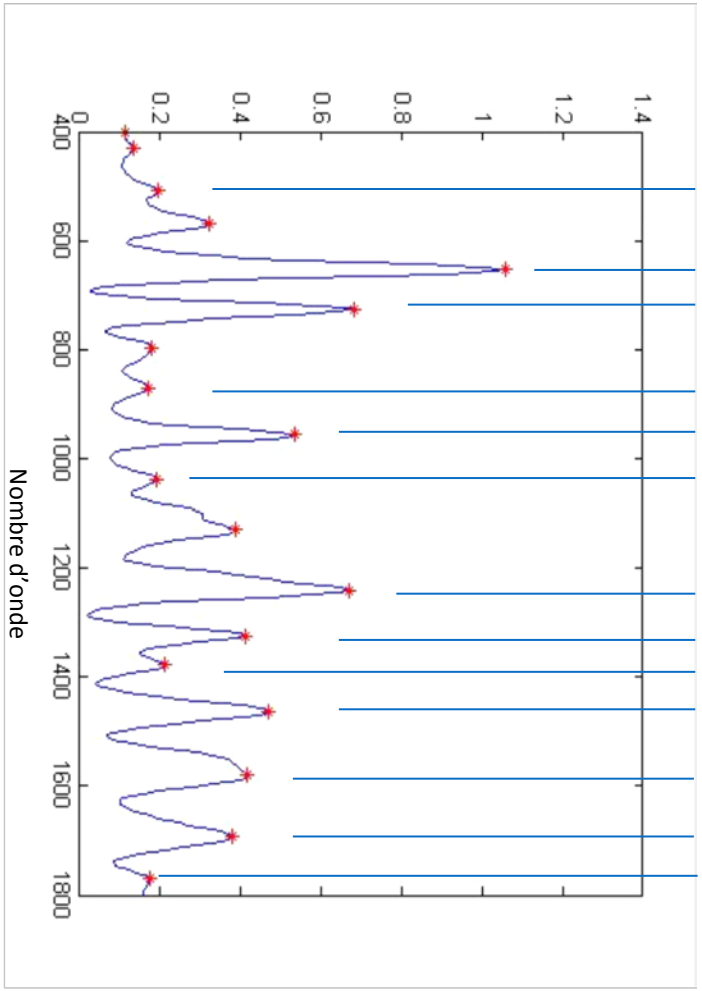
Exemple of E-coli

Lipids

Carbohydrates

Proteins

Nucleic acids



507 : Carbohydrate C-O-C

652 : Tyrosine (Acide Aminé)
727 : Adénine (ADN)

872 : Tyrosine (Acide Aminé)
955: Lipides
1037 : Lipides

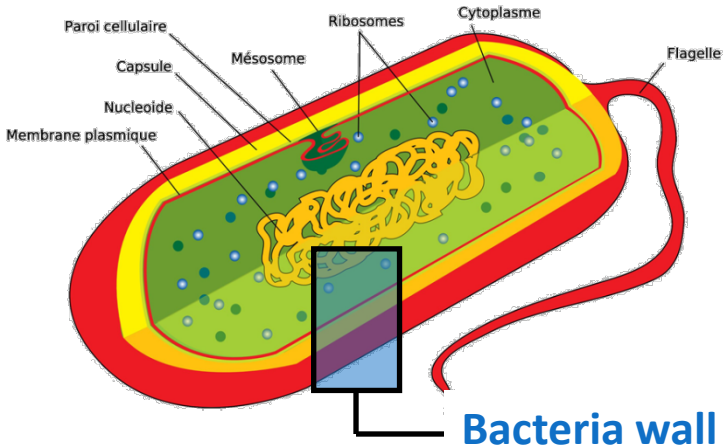
1240 : amide III
1323 : δ(CH2)
1377 : Symm Stretch (CON-), δ(CH2)
1464 : mono-oligosaccharides

1580 : ADN

1771 : Ester

Raman study of bacteria

Allow to distinguish between types of bacteria

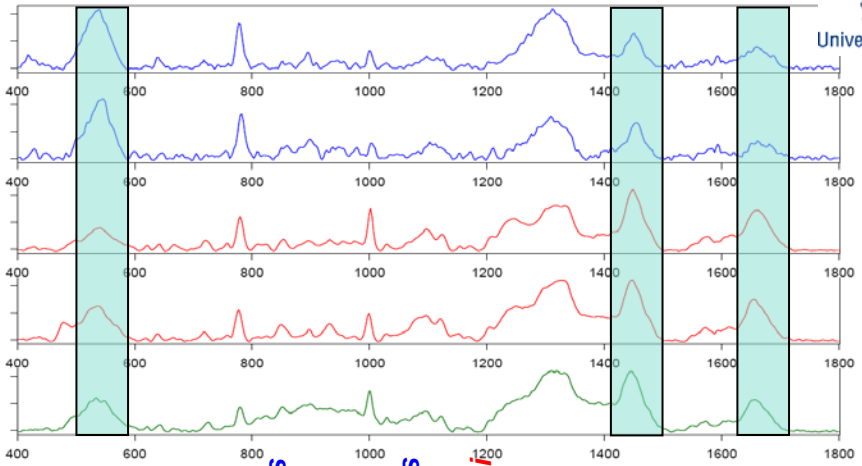


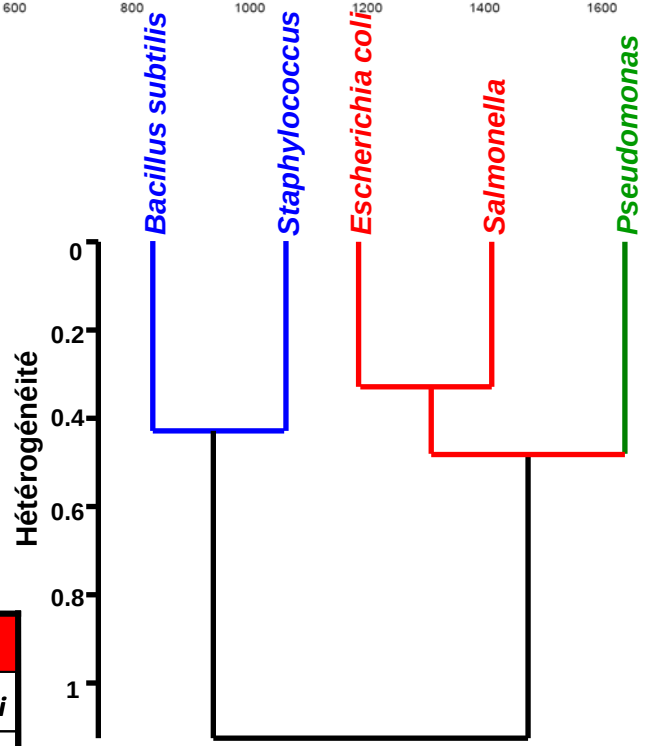
Bacteria wall

0,5 to 3 µm

Type de liaison	Raman shift cm^{-1}
Carbohydrates	460 ; 590
Acides nucléiques	770
Tyrosine	850
Phénylalanine	980 ; 1002
Amide III	1100
Lipides	1240
Amide II	1440
Amide I	1630 ; 1705
Lipides insaturés	1630 ; 1705

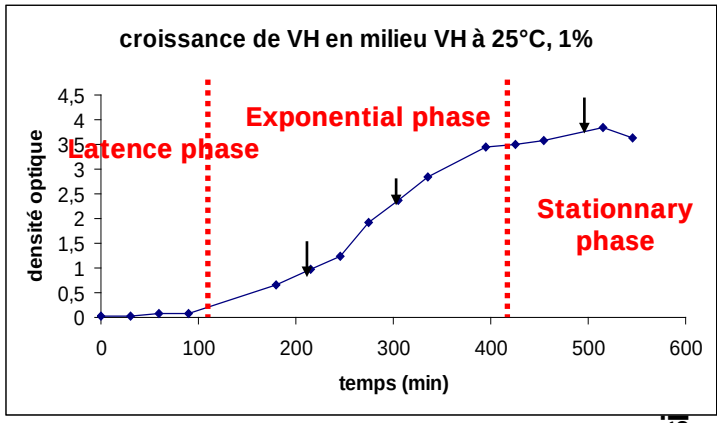
Gram +	Gram-
Bacillus subtilis	*Escherichia coli*
Staphylococcus	*Salmonella*
Streptococcus	*Pseudomonas*



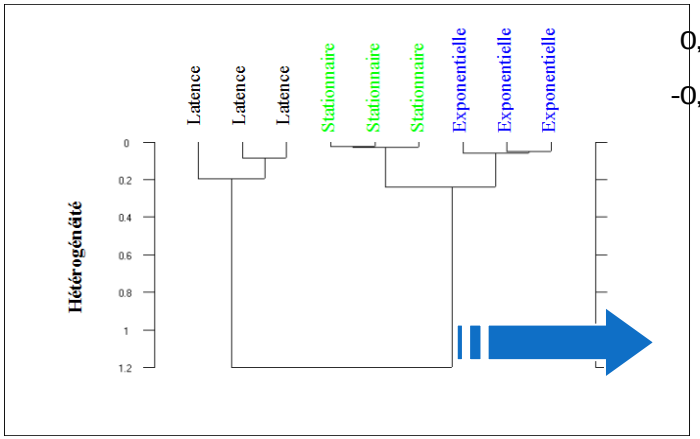


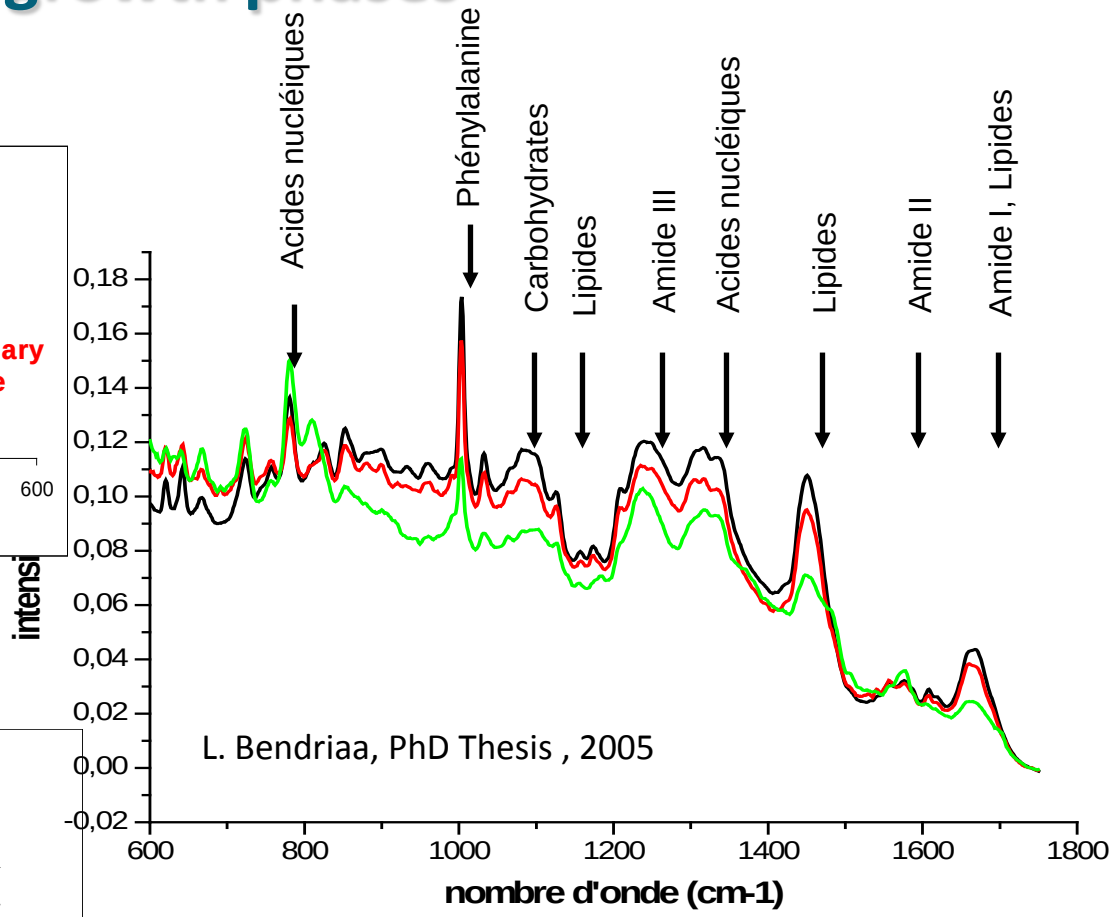
Kengne-Momo, R P; Lagarde, F; Daniel, P et al, Biointerphases –

Raman study of bacteria by Raman spectroscopy vs growth phases



— Latence phase
— Exponential phase
— Stationnary phase





« Rather easy» distinction between young bacteria and old bacteria

Frequency range used for classification:
1450-1750 cm^{-1}

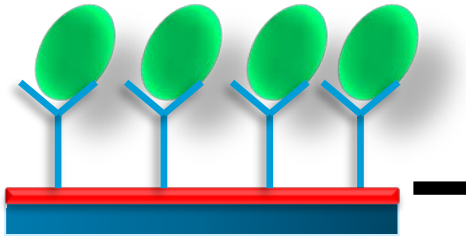
Functionalized surfaces for detection of pathogenic microorganisms

Alternative method

Biosensor based on a « **double check procedure** » :

(1) Specific capture of microorganisms

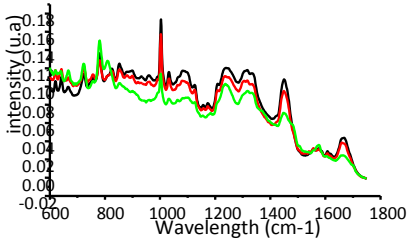
(2) Recognition by Raman spectroscopy



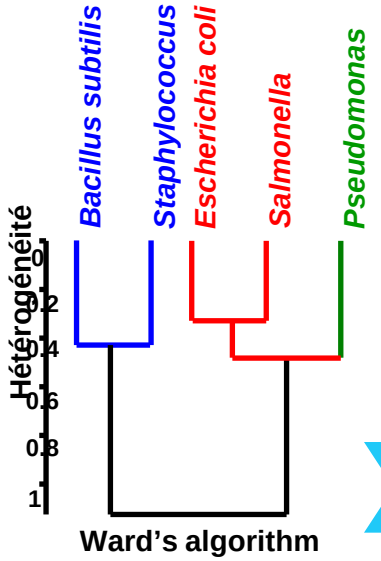
Specific functionalized surface

Raman spectroscopy analysis

Identification via spectra recognition

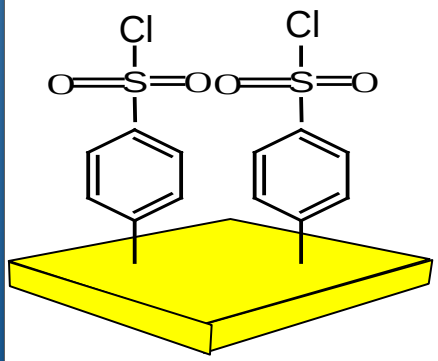


Statistical data analysis

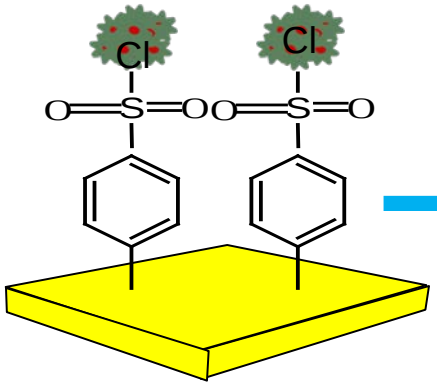


A result of presence/ absence of pathogens in less than 24h

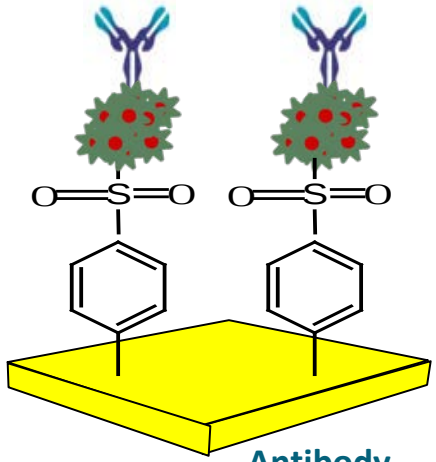
Exemple: Gold surface functionalisation with parabenzenesulfonyle chloride



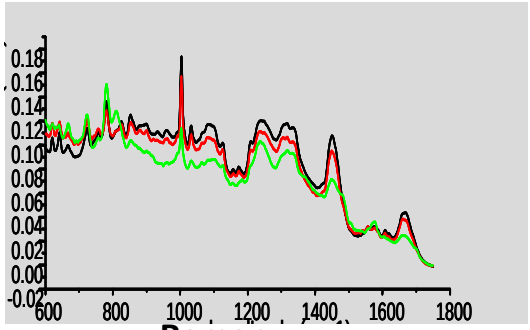
Synthesis of specific surfaces of gold with chemical modifications



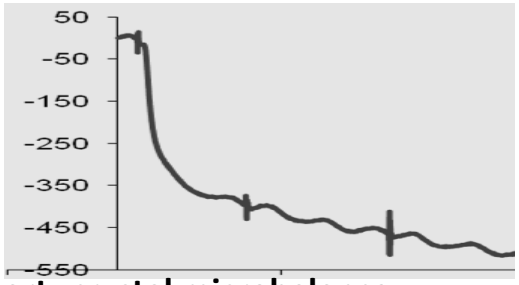
Protein A



Antibody

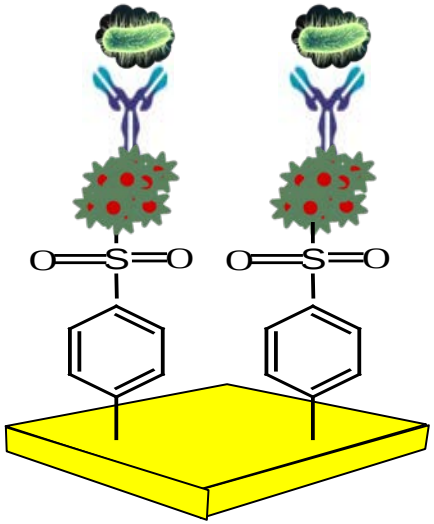


Raman



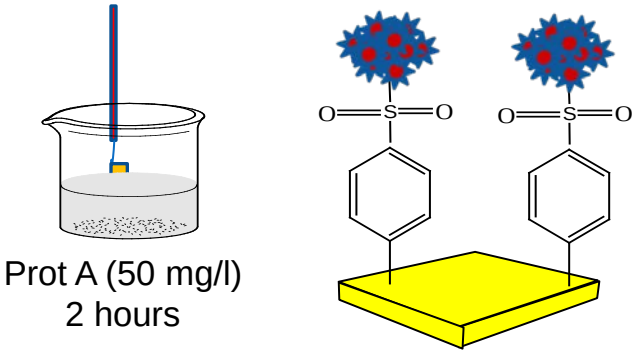
Quartz crystal microbalance

detection



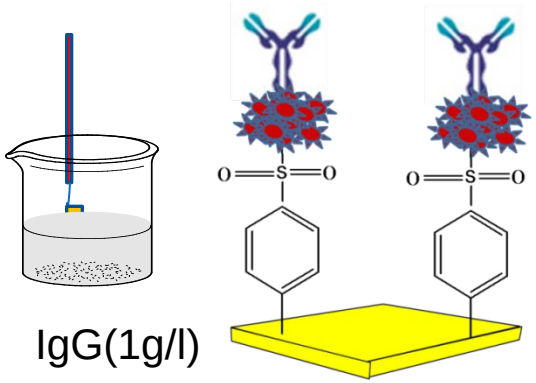
Antibody – antigen specific recognition

Capture of the microorganism



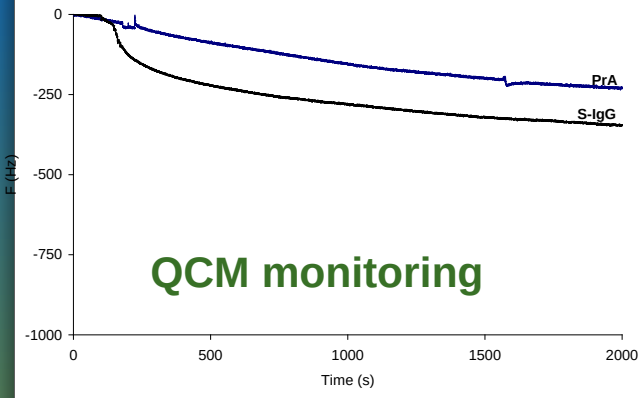
Prot A (50 mg/l)
2 hours

Protein A



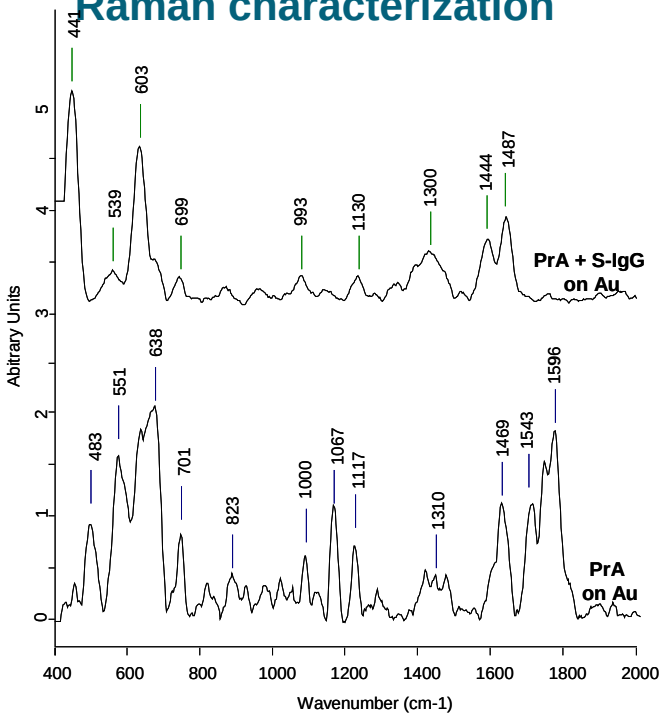
IgG(1g/l)

Antibody

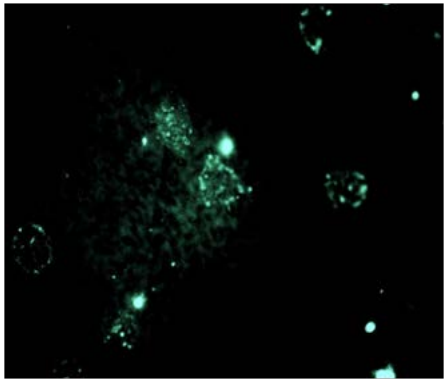


QCM monitoring

Raman characterization



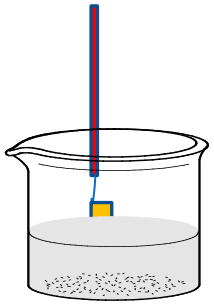
Fluorescence image



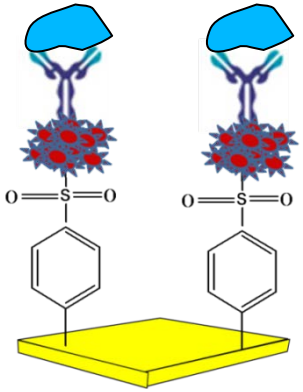
Kengne-Momo, R P ; Daniel, P; Lagarde, F et al
International Journal of Spectroscopy
Article ID 462901 doi:10.1155/2012/462901 (2012)

Capture of the microorganism

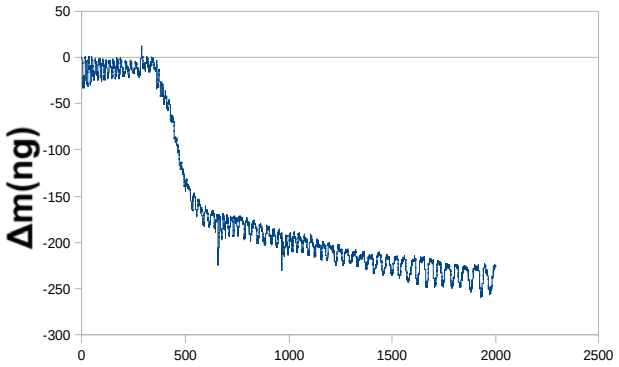
➡ Evidence of the last step of the process



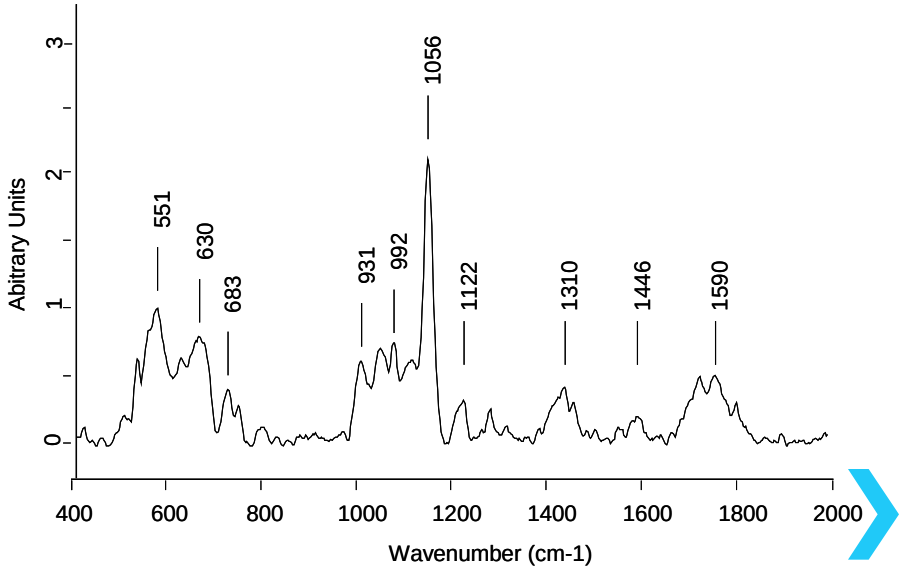
Anti-IgG
(1,07g/l)



QCM monitoring



Raman characterization



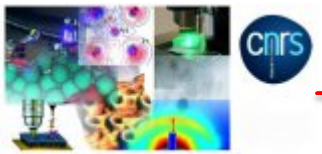
Raman spectra (785 nm, 10 mW) of *Salmonella* immobilized on functionalised Au surface

➢**Functionalization procedure also possible on other type of substrate :**

- Polyethylene traited by plasma
- Functionalized Polyurethane
- Systems including nanoparticles (magnetic, silver, gold: SERS effect)

Conclusion and Perspectives

- Develop a detection kit based on Raman spectroscopy for specific pathogens in food (model and food matrix)
- Target specific resistant bacteria, and try to explore the mechanisms of actions (critical antibiotics)
- Screening of resistant strains along the food chain/environment
- Research at the interface between physics and chemistry of materials



Institute for Molecules and Materials of Le Mans
Department of solid state physics:

- **Physics of advanced materials, Nanomaterials, Surface functionalization**
- **Multiscale and multitime elaboration and characterization technics.**
- **Modeling and simulation.**